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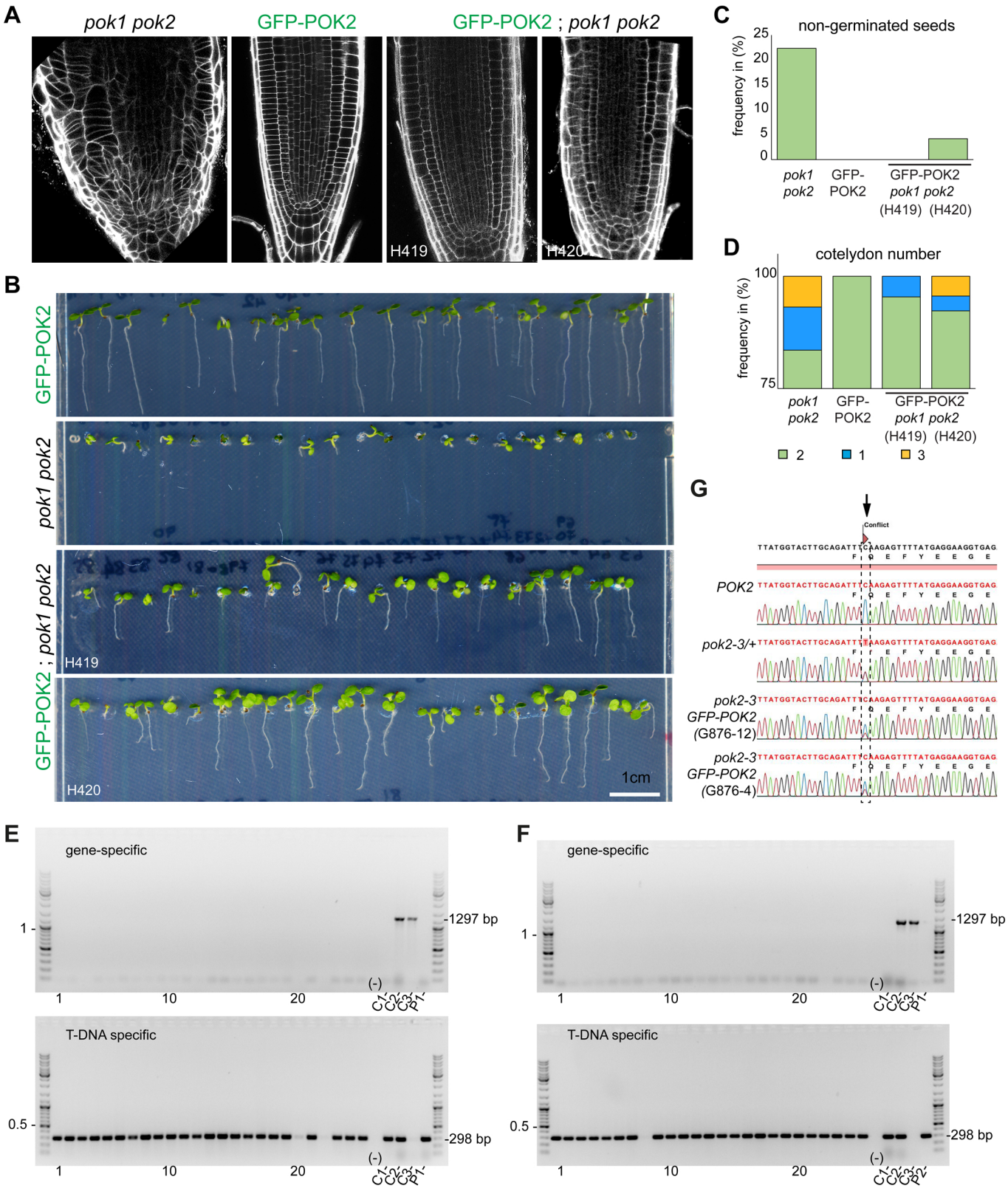
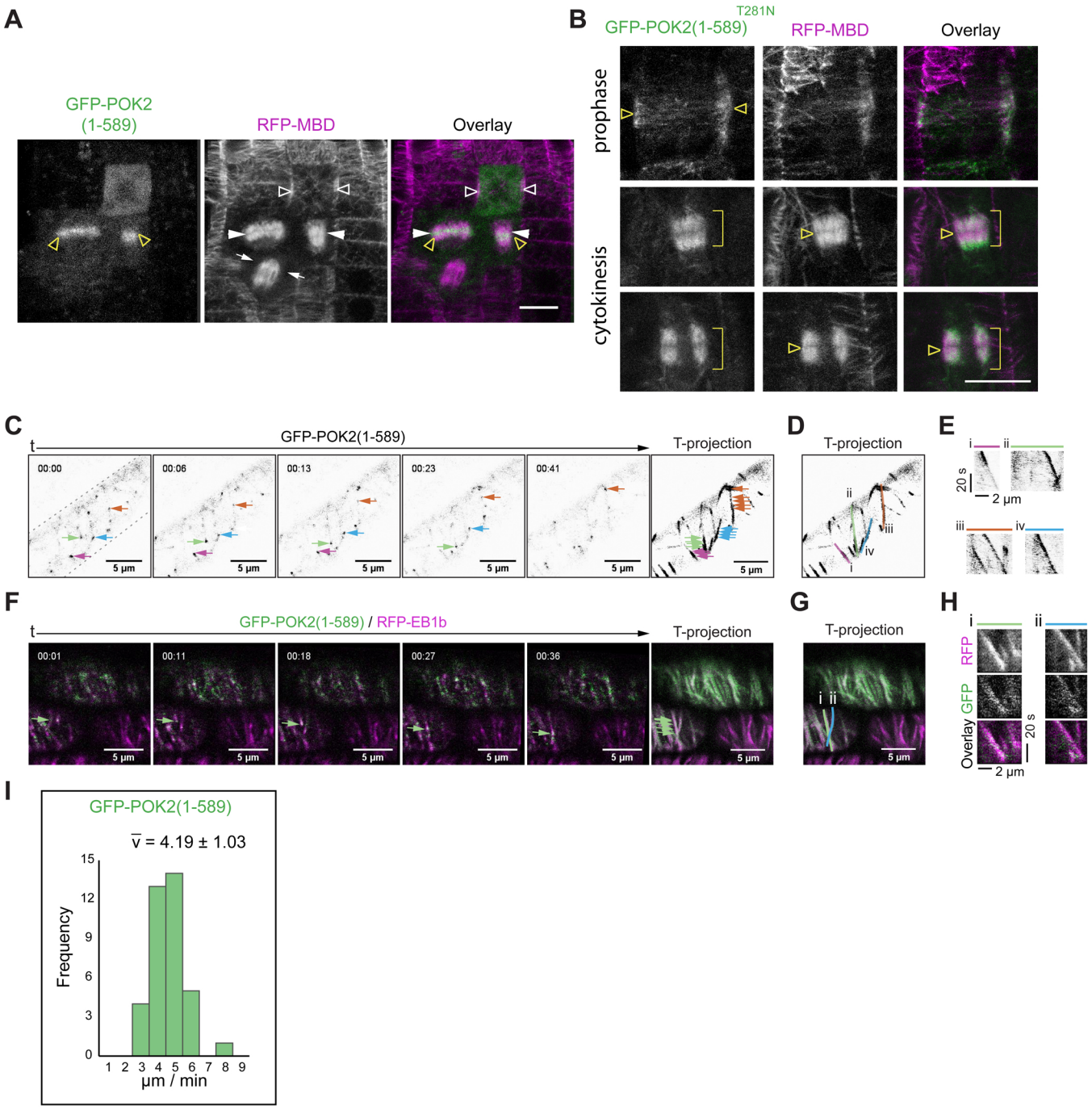
Appendix Content



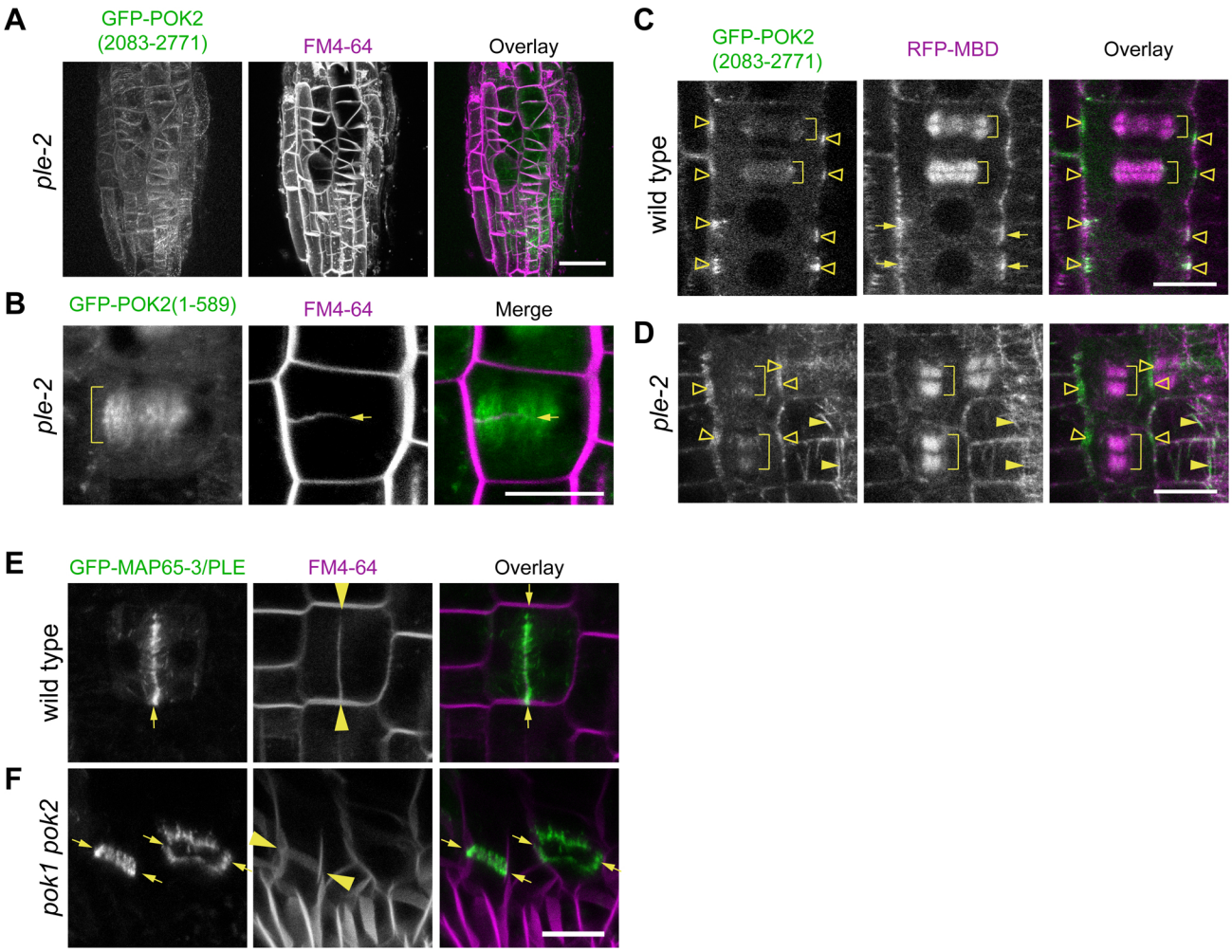
Appendix Figure S1

Appendix Figure S1: Phenotype rescue and localization of GFP-POK2. (A) Single images showing a median plane in roots of *pok1 pok2* seedlings as well as of seedlings expressing GFP-POK2 in wild type and double mutant background (GFP-POK2;*pok1 pok2*, rescue lines) after staining with propidium iodide. Note the restoration of phenotypically wild type cellular organization in the rescue lines (H419, H420). (B) Images of seedlings corresponding to the above mentioned transgenic plant lines. Note the restoration of root growth. (C and D) Quantification of phenotypic abnormalities in *pok1 pok2* mutants, transgenic line expressing *GFP-POK2* (GFP-POK2) and two rescue lines GFP-POK2;*pok1 pok2* (H419, n = 87; H420, n = 95). (C) Frequency (%) of non-germinating seeds. (D) Frequency (%) of seedlings with two (2), one (1) or three (3) cotyledons. (E and F) Representative gel electrophoresis of polymerase chain reaction (PCR) products from 25 randomly selected individuals of (E) H419 and (F) H420 rescue lines. (E) The 298 bp PCR fragment amplified with POK1-Nru/Xba-F and LBb1.3 (Appendix Table S2) indicates the presence of the T-DNA-specific insertion in the POK1 locus. (F) The 1297 bp PCR fragment amplifies from genomic POK1 using POK1-Nru/Xba-F and POK1-Spe-R (Appendix Table S2). For all samples, except for # 20 and 22 in (E) and # 8 in (F), which were of low quality, T-DNA-specific, but not gene-specific PCR fragments were amplified, indicating that the *pok1-1* T-DNA allele was homozygous in the rescue line. C1-C3 indicate controls. C1 *pok1 pok2*, C2 *pok1/+ pok2/+*, C3 Col-0. (-) indicates negative (no DNA) control. P1 is parent G876-4 in (E) and parent P2 G876-12 in (F). *POK1* and *POK2* are closely linked. Therefore, homozygosity of *pok1-1* implies homozygosity of *pok2-3*. (G) The parent plants P1 G876-4 (parent of H419) and P2 G876-12 (parent H420) were sequenced and simultaneous presence of the *pok2-3* mutation and GFP-POK2 transgene were confirmed by the double peaks (dashed box) in the histogram. Thymine (T) indicates *pok2-3* mutation and Guanine (G) indicates presence of *POK2* transgene (also confirmed by Kanamycin resistance). Sequences of wild type and *pok2-3/+* heterozygous plants are shown as well. Substitution of G by Thymine (T) leads to premature STOP codon (Lipka et al., 2014).



Appendix Figure S2

Appendix Figure S2: POK2 motor domain, POK2(1-589) localizes to the phragmoplast midzone. (A) Cells in cytokinesis (yellow triangles, white arrow heads) display GFP-POK2(1-589) signal at the midzone, while cells in prophase (white triangle) and anaphase (white arrows) show cytosolic GFP- POK2(1-589). (B) Co-localization of rigor mutant GFP-POK2 POK2(1-589)^{T281N} with mitotic microtubule arrays in prophase, early and late cytokinesis. The rigor mutant GFP-POK2(1-589)^{T281N} localizes to mitotic (brackets) and cortical microtubules (yellow triangles) point to the preprophase band), but remains excluded from the phragmoplast midzone region (indicated by triangles). Scale bars indicate 10 µm. (C) Individual time points of time series depicting GFP-POK2(1-589) in interphase root cell. Colored arrows point to individual fluorescent dots. In the T-projection of the time series, arrows from individual frames are blotted. (D) T-projection as shown in (C) Tracks (i) - (iv) are indicated by color overlay. (E) Kymographs of tracks (i) - (iv) selected in (H), indicating continuous signal displacement over time. (F) Selected images of a time series of root meristem cells, co-expressing GFP-POK2(1-589) and RFP-End Binding Protein (EB) 1b, arrows indicate GFP-POK2(1-589) signal along RFP-EB1b labelled microtubules. Time-projection (T-projection) shows maximum signal projection of all time frames. Arrows correspond to arrows in single time frames. (G) T-projection of time series in (F), green (i) and blue (ii) line indicate line selections, used for kymographs depicted in (H). (H) Single channels and overlay of kymographs along line selection indicated in (G). Note that the signal contrast edge of GFP-POK2(1-589) and RFP-EB1b co-align. (I) Frequency distribution of *in vivo* speeds of GFP-POK2(1-589) (n= 37, combined from five cells in four root meristems). Velocity 4.19 ± 1.03 µm / min is presented as mean ± STDV. Relates to Figure 4 and Movie EV3, Movie EV4.



Appendix Figure S3

Appendix Figure S3: Genetic interaction of POK2 and MAP65-3/PLEIADE. (A) Root meristem of *pleiade* (*ple-2*) mutant expressing GFP-POK2(2083-2771). FM-64 staining allows visualization of the plasma membranes. Scale bar indicates 25 µm. (B) GFP-POK2(1-589) motor domain along phragmoplast microtubules (brackets) in the *pleiade* (*ple-2*) mutant. Arrow points to a cell wall stub, characteristic for *ple* mutants. Scale bar indicates 10 µm. (C and D) GFP-POK2(2083-2771) localization in (C) wild type and (D) *ple-2* mutant. GFP-POK2(2083-2771) co-localize with microtubules (brackets, arrows and arrowheads) and accumulates at the cortical division site (triangles). Scale bars 10 µm. (E and F) GFP-MAP65-3/PLE localizes at the midzone (arrows) in (E) wild type and (F) *pok1 pok2* mutant cytokinetic cells. Plasma membranes visualized by FM4-64 staining. Arrow heads indicate cell plate fusion sites. Images are maximum Z-projections. Scale bars indicate 10 µm.

Appendix Table S1: Oligonucleotides for Cloning

Cloning	**Name**	**Oligos 5‘ – 3‘**
MAP65-3/PLE	PLE_ATG_KPN_F	atggtaccATGGCAAGTGTTCAAAAAGAT
	PLE_w/o STOP_NOT_R	atgcggccgcttAACCAAACGACATTCAGACTGTA
pENTR:POK2 (1-189)	NotI_POK2_ATG_F	AATAATAACATGCGGCCGCaATGTCAAAGGAGACCAAGC TTTC
	POK2MD1-189aa_R	aaTCTAGAtcaCCAGAAAGATGGATCTTCCTT
pENTR:POK2MD(183-589)	NotI_POK2_549_F	AATAATAACATGCGGCCGCaATGGAAGATCCATCTTTCTG GATGGATCACAA
	Asc_POK2_1770 stop R	AATAATAACATGGCGCGCCtta*ACTTGATGGCGAATCGAC T*
pENTR:POK2(1-589)	NotI_POK2_ATG_F	AATAATAACATGCGGCCGCaATGTCAAAGGAGACCAAGC TTTC
	Asc_POK2_1770 stop R	AATAATAACATGGCGCGCCtta*ACTTGATGGCGAATCGAC T*
pENTR:POK2(1-589)^{T281N}	POK2_T281N_F	AAGTGGAAAGaacTATACAATGCTT
	POK2_T281N_R	AAGCATTGTATAgttCTTTCCACTT
pDONR221:POK2(2083-2771)	minB1F-POK2C	aaaaagcaggctccaccATGGACAAGAAAGATGAAATAAAGG AAATC
	minB2R-POK2C stop	agaaagctgggtcCTACCTGTCTAAAGAAGAGAAAAAAGGA AC
pENTR:POK2(2083-2771)	KAT2_BamHI_6250_F	ggatccGACAAGAAAGATGAAACAAAGGAAATC
	POK2_STOP_XbaI_R	ATtctagaTTtcacctgtctaaagaagagaaa
pENTR:POK2(Δ590-2082) and pENTR:POK2(1-2771)	POK2 MD linker C_F	aagttcctatactttctagagaataggaacttcAGGGACAAGAAAGA TGAAACAAA
	POK2 MD linker C_R	tcctattctctagaaagtataggaacttcccTACACTTGATGGCGAA TCGA
PCRI (6847 bp genomic fragment)	Kat2-BsrGI-F	ccgcggcatgctgtacaagagtgatatcg
	Kat2-68491R	gactctgactccatgatcttc
PCRII (5464 bp genomic fragment)	POK2-799 F	ATGGAGAGGAGTATAAATGGGTA
	Kat2-EcoRI-R	ctgtccttcactacagtgggctggag
	B1F	ggggacaagtttgtacaaaaaagcaggctccacc
	B2R	ggggaccactttgtacaagaaagctgggtc
pENTR:EB1b	EB1b ecoRI F	gaattcAAAAATGGCGACGAACATT
	EB1b xhoI R	ctcgagTTAAGTTTGGGTCTCTGCAGCA
Genotyping		
POK1-XbaI/NruI-F	*POK1/pok1-1*	gctctagatcgcgacagcattgacaagaatc
POK1SpeI-R	*POK1*	tcactagtgcacctctatcatag
LBb1.3	*pok1-1*	attttgccgatttcggaac

Appendix Table S2: Oligonucleotides for Transcript amplification

Fragment	Primer pair	Oligos 5'–3'
1	GFP-CDS 467_F	acaagcagaagaacggcatcaa
	POK2-BstEII_R	ttaggcggtcaccctcagcgc
2	NotI-POK2_ATG_F	aataataacatgcggccgcaatgtcaaaggagaccaagctttc
	POK2-BstEII_R	ttaggcggtcaccctcagcgc
3	POK2-BstEII_F	gcgctgagggtgaccgcctaa
	POK2-BsrGI_R	ccgcggcgatatcactcttgtacagcatg
4	POK2-BsrGI_F	ccgcggcatgctgtacaagagtgatatcg
	POK2-BspHI_R	actagtgagatagcacaatccttcatgat
5	POK2-BspHI_F	actagtatcatgaaggattgtgctatctc
	POK2-5084_R	cctctcatcgatggcatcat
6	POK2-5084_F	atgatgccatcgatgaga
	POK2-BamHI_Stop	ggatcctttcacctgtctaaagaagagaaa

Appendix References

Lipka, E., Gadeyne, A., Stöckle, D., Zimmermann, S., De Jaeger, G., Ehrhardt, D.W., Kirik, V., Van Damme, D., and Müller, S. (2014). The Phragmoplast-Orienting Kinesin-12 Class Proteins Translate the Positional Information of the Preprophase Band to Establish the Cortical Division Zone in Arabidopsis thaliana. Plant Cell **26,** 2617-2632.